Remarks

Claims 1-20 are pending. Claims 10-20 were previously withdrawn in response to a restriction requirement. In this paper, claim 1 is amended, claim 3 is cancelled and new claims 21-25 are added. Accordingly, claims 1-2, 4-9 and 21-25 are presented for examination.

In the Office Action dated January 31, 2007, claim 3 is objected to as being dependent upon a rejected base claim. The examiner has indicated that claim 3 would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

Claims 1-9 stand rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1-9 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Suzuki et al., U.S. Patent No. 6,803,226 in view of Inoue, Dersent-Acc-No. 2000-596789, and Murao, JP60149379.

Rather than amend claim 3, applicant has amended claim 1 to include the limitations of claim 3. Claim 1 has been further amended to include the biological names of the mushroom species originally recited in claim 3. Claim 1 is allowable as amended.

Claim 3 has been cancelled.

As described in the specification and recited in the claims as amended, the present invention relates to methods for producing an edible mushroom-producing fungi containing biologically active forms of folic acid. A growth environment for edible mushroom-producing fungi is provided comprising a substrate, such as brown rice, and water. As recited in claim 1 as amended, a synthetic folate is added to the growth environment and a spawn of a mushroom producing fungi selected from the group consisting of Lentinola edodes, Ganoderma lucidum and Grifola Frondosa is combined with the substrate. The fungi is cultivated for a sufficient time to permit the mushroom-producing fungi to accumulate a nutritionally significant amount of methylated folate.

Claims 2 and 4-9 depend from claim 1 and recite additional limitations regarding the substrate and growth environment, and the processing of the fungi after cultivation to produce a product for consumption as a nutritional supplement.

Applicant has added new claims 21-25. New claim 21 depends from claim 1 and recites that the concentration of synthetic folate in the growth environment is about 400 mg/ml. Support for this limitation can be found at paragraph 0029.

New claim 22 recites the method for producing an edible mushroom-producing fungi containing biologically active forms of folic acid as recited in claim 1, but instead using a species selected from the group consisting of Cordyceps sinesis (cordyceps), Tremella fuciformis (white wood ear), and Coriolus versicolor (Turkey tail). These are all species of edible mushrooms, and they are therefore within the scope of the description and claims as filed. New claims 23-25 depend from claim 22 and define further embodiments of the method. No new matter is added.

The methods of the present invention result in fungi having an increased quantity of biologically active methylated folates. The fungi can be eaten directly or used in nutritional supplements to provide the biologically active methylated folates to an individual.

Rejection of Claims 1-9 Under 35 U.S.C. §112, Second Paragraph

Claims 1-9 stand rejected under 35 U.S.C. §112, second paragraph as being indefinite. The examiner states that the term "spawn" and the phrase "combining a spawn of at least one edible mushroom-producing fungi with the substrate" would be ambiguous to one of ordinary skill in the art. Applicant respectfully disagrees with the examiner, as these are common terms well understood by one skilled in the art.

"Mushroom spawn" is commonly understood to mean "the mycelium, or primary filamentous growth of the mushroom." Webster's Revised Unabridged Dictionary (1996). "Mycelium" means "the mass of hypae that form the vegetative part of a fungus." As described in the Fact Sheet, "Shiitake Mushroom Production" published by The Ohio State University (copy attached), "Active fungal cultures intended as inoculum for mushroom cultivation are called spawn." As these references demonstrate, those skilled in the art understand the meaning of the term "spawn" in relation to mushroom cultivation.

One skilled in the art would also readily understand the meaning of "combining a spawn . . . with the substrate." In order to cultivate mushrooms, the spawn must be introduced or combined with the substrate. Accordingly, claim 1 as amended clearly defines the metes and bounds of the claim, and claim 1 meets the requirements of 35 U.S.C. §112, second paragraph.

The limitations of claim 3 have been incorporated into claim 1, and claim 3 has been cancelled. In amendment claim 1, the biological names for the various mushroom species have been used as suggested by the Examiner.

Rejection of Claims 1-9 Under 35 U.S.C. §103(a)

Claims 1-9 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Suzuki et al., U.S. Patent No. 6,803,226 in view of Inoue, Dersent-Acc-No. 2000-596789, and Murao, JP60149379.

As noted by the Examiner, claim 3 of the application was allowable if rewritten to include all limitations of the claims from which it depends. Applicant has amended claim 1, from which claim 3 depended, to include the limitations of claim 3. Accordingly, claim 1 and the claims depending from claim 1 are now in condition for allowance. Applicant provides the following arguments for patentability of the claims as amended and the new claims added in this paper.

Suzuki describes method of forming an artificial Shiro of Matsutake, a species of mushroom-producing fungi, by culturing Matsutake hyphae in a culture substrate containing a substance capable of controlling the cell membrane permeability of the hyphae and enhancing the hydrophilic property of the hyphae. Col. 2, lines 12-23. As described by Suzuki, Matsutake is unlike other mushroom producing fungi in that the production of Matsutake requires the root of a living tree. An assembly of hyphae called a "Shiro" forms where the Matsutake mushrooms grow in the root. Col. 1, lines 22-29. Suzuki is directed to methods of producing an artificial Shiro of Matsutake mushrooms to increase production of Matsutake mushrooms.

The methods described by Suzuki are specifically directed to one species of mushroom, Matsutake, to address the particular problems associated with cultivating that species. The methods of Suzuki are not directed or intended for use with the species of mushrooms recited in claim 1 as amended or in new claim 21.

Suzuki generally teaches the use of a surfactant and vegetable oil in creating the artificial Shiro for growth of Matsutake mushrooms using soil or soil with vermiculite as a substrate for use in cultivating the mushrooms. Col. 6, lines 30-38. Suzuki does not recognize, teach or suggest the use of added synthetic folates in the growth environment to produce mushrooms having a nutritionally significant amount of methylated folic acid. The Examiner has cited Table

1 of Suzuki as describing addition of folic acid to the growth medium for the mushrooms. In fact, Table 1 describes a medium used to prepare an inoculum, or spawn, of Matsutake hyphae. The hyphae is then cultured in one of the media described in Suszuki at Col. 9, line 63 to col. 11, line 33. None of those media include any folic acid or other folate additive.

Moreover, even in the media used to prepare the inoculum of Matsutake, the amount of folic acid is minimal, and is not nearly sufficient to produce the mushrooms described in the present application. As described in Table 1, 10 ml of a vitamin solution containing 3 mg of folic acid/1000 ml (i.e. 0.003 mg/ml folic acid) of vitamin solution is added is added to 1000 ml of the medium. Therefore, the medium contains 0.03/1000, or 0.00003 mg of folic acid. Even if Suzuki described the use of this solution in cultivating the mushrooms, which he does not, the amount of folic acid in the solution is insufficient to provide mushrooms having a nutritionally significant amount of methylated folic acid as recited in the claims as amended. Moreover, this is plainly less than the amount of folic acid recited in new claim 21 and 24.

The additional references cited by the Examiner do not address these deficiencies in Suzuki. The abstract of Inoue, Derwent-Acc-No 2000-596789 generally describes the use of brown rice with sawdust, rice bran, wheat and rye as a substrate for cultivating undisclosed species of mushrooms. Inoue does not describe the species of mushrooms recited in claim 1 as amended or in new claim 22. Suzuki describes only the use of soil or soil with vermiculite as a substrate for the mushrooms. Suzuki does not teach or suggest the use of brown rice as a substrate. Because Suzuki was attempting to provide a speciailized growth media for a particular species of mushrooms, the result of changing the substrate from the particular substrate described by Suzuki to another substrate would not be predictable. Accordingly, one skilled in the art would not be motivated to replace the soil taught by Suzuki with brown rice as a substrate as recited in the amended claims.

Moreover, Inoue does not describe at all the addition of folic acid to the growth medium, much less describe the addition of sufficient folic acid to result in a nutritionally significant amount of folic acid in mushrooms as recited in the claims as amended. Accordingly, the combination of Suzuki and Inoue does not result in a method meeting all of the limitations of the claims as amended.

Finally, Murao, JP 60149379A, describes only the use of p-aminobenzoic acid in a growth medium for mushrooms. Murao, like Inoue, does not describe at all the addition of folic

acid to the growth medium, much less describe the addition of sufficient folic acid to result in a nutritionally significant amount of folic acid in mushrooms as recited in the claims as amended. Accordingly, the combination of Suzuki, Inoue and Murao does not result in a method meeting all of the limitations of the claims as amended.

The pending claims are believed to be allowable over the prior art of record for at least the reasons set forth above. Accordingly, it is respectfully requested that this application be allowed and a Notice of Allowance issued. If the Examiner believes that a telephone conference with Applicants' attorney would be advantageous to the disposition of this case, the Examiner is cordially requested to telephone the undersigned. If the Examiner has any questions in connection with this paper, or otherwise if it would facilitate the examination of this application, please call the undersigned at the telephone number below.

A petition for a three month extension of time and the associated fee have been submitted herewith. No additional fees are believed to be required. In the event the Commissioner of Patents and Trademarks deems additional fees to be due in connection with this application, Applicant's attorney hereby authorizes that such fee be charged to Deposit Account No. 50-3569.

Respectfully submitted,

Date: July 31, 2007

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FactSheet Extension

Shiitake Mushroom Production:

Obtaining Spawn, Obtaining and Preparing Logs, and Inoculation

Stephen M. Bratkovich, formerly of Ohio State University Extension.

In nature, the shiitake fungus propagates and spreads from spores produced by the mushroom. However, for cultivation, spore germination is too unreliable. Instead, logs are inoculated with actively growing fungus. The fungus is first adapted to wood by growing it directly on small pieces of wood. Active fungal cultures intended as inoculum for mushroom cultivation are called spawn. Because the quality of the crop can be no better than the spawn, growers must use viable shiitake spawn of a good variety in pure culture, free of weed fungi and bacteria.

Spawn Strain Characteristics

Different cultivars or strains of shiitake may perform differently under different conditions. Initially, growers should try more than one strain to ensure success. Also, growers can extend the growing season by using strains that fruit under different environmental conditions. For example, a grower in southern Ohio could use a cold-weather strain for spring and fall production, and a heat-tolerant strain for summer production.

The following strain characteristics need to be considered when ordering spawn from commercial suppliers:

- Preference for type of wood
- Resistance to weed fungi
- Speed of colonization (time of first fruiting)
- Ease of fruiting
- Season of fruiting
- Ability to stimulate (force) fruiting
- Required temperature for fruiting
- Size, shape, color and flavor of mushrooms
- Mushroom storage characteristics

The individual grower will need to experiment with different strains and decide which work best in a particular situation.

Sources of Spawn

There are numerous spawn suppliers throughout the United States and Canada. In addition to experimenting with different strains, growers are encouraged to purchase spawn from more than one spawn supplier. Many spawn suppliers also sell equipment and supplies related to mushroom growing; product catalogs are often available from them upon request. Fact Sheet F-39 Shiitake Mushroom Production: Introduction and Sources of Information and Supplies, includes an up-to-date list of spawn suppliers. It is available from your county office of Ohio State University Extension.

Forms of Spawn

Shiitake spawn can be purchased on two mediums: sawdust and wood dowels. It is usually supplied in either sealed plastic or glass containers. The spawn should be moist, white (sometimes with a brown crust) and appear rather fuzzy. Good quality spawn smells mushroom-like, not mildewy or mold-like. Weed fungi and bacteria are controlled by not damaging or opening the spawn container until use of the entire contents.

Spawn Storage

Spawn must be kept away from direct sunlight and temperature extremes. Storage for a month or more should be in a cool (34-38 F) location away from direct sunlight. Spawn must not be frozen. Prior to inoculation spawn should be warmed to room temperature (70 F) for two to five days.

Obtaining and Preparing the Logs

Trees cut for shiitake mushroom production should be harvested as part of an overall forest management plan. Individuals interested in producing shiitake mushrooms from their woodlot should contact a forester for assistance in selecting the appropriate trees.

Suitable Tree Species

The hardwood tree family most recommended in the United States for shiitake cultivation is the beech family (Fagaceae). The particular genus most successful in this family is Quercus (oak). All oak trees can be used with the possible exception of live oak. The thicker bark oaks such as white and chestnut oak are often preferred over the thinner bark red, scarlet and pin oak.

Beech, birch, chestnut, chinkapin, alder, maple, cottonwood, willow, aspen, poplar, elm and hophornbeam are suitable species but may have commercial limitations. As a rule, the thin-barked low-density species provide relatively quick mushroom production but only for a short time period. Locust, walnut and all conifers are not suitable for shiitake cultivation.

Sweet gum (Liquidambar styraciflua) and sycamore (Platanus occidentalis) have outperformed oak species in selected trials in North Carolina. Other species may prove to be excellent for shiitake cultivation once testing is completed.

Tree Quality

Logs used for shiitake production must be cut from live, healthy trees. Living trees with obvious insect or disease damage should not be used.

Optimum log size is 4-8 inches in diameter and 3-4 feet in length; standard lengths make operations much more convenient. Logs with a thick sapwood layer and small heartwood area are preferred. Logs can be cut from young hardwood trees or branches of older trees.

Logs should be straight for ease in handling but crooked logs can be used. A smooth bark will make the inoculation (seeding) process easier but thin bark tends to crack and peel sooner than thick bark. Regardless of thickness, the bark must be intact on the log.

Tree Felling

Some authors suggest the optimum felling time is when 30 to 80 percent of the tree leaves on the chosen species have changed color. Others suggest tree felling during the coldest time of winter. One reason for the different opinions on tree felling is due to the different theories on the best time to inoculate (seed the logs). Most shiitake growers and researchers agree, however, that trees should be felled sometime during the dormant season (mid-autumn to late winter), before spring sap movement and bud swell.

Logs should not be used for shiitake production if they were cut from felled trees that seasoned during the summer months. For example, trees cut prior to the summer months (even if cut while dormant) should not be used after being exposed to the warm weather conditions of summer.

Log Preparation

After tree felling, logs need to be prepared for inoculation. Regardless of the method of log preparation, two areas need careful attention: the moisture content of the log needs to be maintained above 35 percent and potential log contaminants must be minimized.

One method of reducing moisture loss is to keep logs in whole tree lengths and cut to final log size prior to inoculation. Rain and snowfall can be permitted to wet the logs. If trees must be immediately cut to final log length, logs should be protected from drying winds and direct sunlight by covering with burlap, shade cloth or plastic. If possible, logs should be stacked firewood-style in full shade under conifers. Watering or soaking logs is recommended several days before inoculation if the moisture content drops below 35 percent. Log surfaces should be allowed to dry prior to inoculation.

Log contaminants (insects, diseases, etc.) can be reduced by storing the logs off the ground. Tree length logs can have the butt supported on the stump. Logs cut to length can be placed on pipe, concrete blocks or other suitable material that will keep them off the ground. If stacking logs in contact with the ground, select a well-drained site with good air circulation and use cull logs as supports.

When logs are cut to final length, plan all cuts to give the most good logs. All diseased and wounded sections, forks, crotches and major kinks should be cut out. Small branches should be removed from logs, leaving a stump of approximately two inches. A wire brush can be used to remove lichens and moss from the bark prior to inoculation.

Inoculation

Inoculation is the introduction of the live shiitake fungus into the log. A one-time inoculation will produce mushrooms after 6-18 months and will continue to produce for 3-6 years.

In the past, logs were normally cured after felling for at least two weeks before inoculation. However, many researchers and spawn suppliers are now

recommending inoculation as soon as possible after felling. Also, early spring inoculation is now being replaced by fall and spring inoculation. Inoculation should always be done in a shaded area to avoid direct exposure of the spawn to sunlight.

Logs should be watered if the internal log moisture content drops below 35 percent prior to inoculation. Occasional thorough waterings are better than frequent light waterings. The former will increase the internal log moisture content while the latter often just wets the bark surface.

Personnel, Equipment and Supplies Needed

While the entire inoculation process can be done by one person, a minimum of three is suggested: one person to drill holes, one to place the spawn in the holes and one to seal the inoculation sites. A fourth person can be useful in moving logs from one work station to the next.

In addition to the spawn, equipment and supplies needed for inoculation are: drill (preferably high speed if many logs are to be inoculated), bits, work table or saw buck, yard stick or measuring tape, hammer (for dowel spawn), inoculation tool (optional, for sawdust spawn), paraffin/cheese wax/plastic foam (for sealing inoculation sites), heat source (for melting wax), wax dropper or brush (for applying wax), and rubbing alcohol.

Inoculation Procedure

Logs to be drilled should be secured in a saw buck or similar arrangement to prevent the logs from moving (Figure 1). The log bark should be free of dirt and other possible contaminants. Dip the drill bit in rubbing alcohol after finishing each log as a precaution.

The hole-drilling pattern will vary from grower to grower. A general recommendation is to space holes 6-16 inches within rows and 2-4 inches between rows since the shiitake mycelium runs well with the grain but poorly across the grain. Adjacent rows should be offset from one another to create a diamond pattern on the log (Figure 2).

Depth and diameter of the holes will depend on the source as well as the form of spawn. Hole depth generally ranges between 3/4 and 11/4 inches with hole diameter between 1/4 and 1/2 inch. Most spawn suppliers will recommend dimensions for hole size. After a log has been drilled, holes should be filled immediately so as not to lose moisture or permit entry of airborne spores.

Hands should be washed and then wiped with rubbing alcohol before handling spawn. On a work table or second saw buck, dowel spawn can be placed into the holes and then gently pounded in with a hammer. A convenient method is to initially hold the dowels with forceps. Sawdust spawn can either be inserted manually or with an inoculation tool available from many spawn suppliers. Disagreement exists as to whether the sawdust spawn should be packed tightly in the hole or just lightly tamped in. Growers are advised to follow the spawn supplier's recommendations.

The final step in the inoculation process is to seal the spawn-filled hole with either a paraffin or cheese wax or styrofoam plug. Holes are sealed to prevent loss of moisture, to prevent contamination by undesirable microorganisms and to allow the spawn to grow within the confines of the log. Hot wax also tends to disinfect the inoculation surface. Melted wax can be applied by brush or wax dropper (similar to a turkey baster). Styrofoam plugs are placed on top of the spawn, flush with the surface of the bark.

Suggested Practices After Inoculation

All inoculated logs should be coded to record important information such as spawn strain, tree species, etc. Small aluminum tags fastened to log ends with a staple work well for this purpose. Good record keeping will enable growers to duplicate successful practices by learning from past experiences.

Inoculated logs may be dead piled (firewood style) and shaded with plastic immediately following inoculation. If the log moisture content is low, burlap or similar material should be used to allow rain to reach the logs. If surface molds develop, logs should be moved from temporary to a permanent laying position.

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Figure 1. Log drilling in a saw buck. Ohio State University Extension

Figure 2. Pattern to guide the placement of holes (inoculation sites) across the log surface.

Keith L. Smith, Associate Vice President for Ag. Adm. and Director, OSU Extension.

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1 result for: *mushroom spawn*

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Mushroom spawn

Mushroom \Mush"room\, a. 1. Of or pertaining to mushrooms; as, mushroom catchup.

2. Resembling mushrooms in rapidity of growth and shortness of duration; short-lived; ephemerial; as, mushroom cities.

Mushroom anchor, an anchor shaped like a mushroom, capable of grasping the ground in whatever way it falls.

Mushroom coral (Zo["o]l.), any coral of the genus Fungia. See Fungia.

Mushroom spawn (Bot.), the mycelium, or primary filamentous growth, of the mushroom; also, cakes of earth and manure containing this growth, which are used for propagation of the mushroom.

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my·ce·li·um [mahy-**see**-lee-*uh*m] Pronunciation Key - Show IPA Pronunciation

–noun, plural –**li·a** [-lee-*uh*] Pronunciation Key - Show IPA Pronunciation. *Mycology.*

the mass of hyphae that form the vegetative part of a fungus.

[Origin: 1830–40; < NL, equiv. to Gk *myk*- MYC- + (*h*)*ēlos* wart, nail + NL -*ium* -IUM]

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my·ce·li·um (mī-sē'lē-əm) Pronunciation Key

n. *pl.* **my·ce·li·a** (-lē-ə)

1. The vegetative part of a fungus, consisting of a mass of branching, threadlike hyphae.
2. A similar mass of fibers formed by certain bacteria.

[New Latin : **myc(o)-** + Greek hēlos, *wart.*]

my·ce'li·al (-lē-əl) *adj.*

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mycelium

noun

the vegetative part of a fungus consisting of a mass of branching threadlike hyphae

The American Heritage Science Dictionary – *Cite This Source*

mycelium (mī-sē'lē-əm) Pronunciation Key

Plural **mycelia**

The mass of fine branching tubes (known as **hyphae**) that forms the main growing structure of a fungus. Visible structures like mushrooms are reproductive structures produced by the mycelium.

American Heritage Stedman's Medical Dictionary – *Cite This Source*

my·ce·li·um (mī-sē'lē-əm)

n. pl. **my·ce·li·a** (-lē-ə)

The vegetative part of a fungus, which consists of a mass of branching, threadlike hyphae.

my·ce'li·al or **my·ce'li·an** *adj.*

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Pronunciation: mI-'sE-lE-&m
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Inflected Form: *plural* **my·ce·lia** /-lE-&/
: the mass of interwoven filamentous hyphae that forms especially the vegetative body of a fungus and is often submerged in another body (as of soil or organic matter or the tissues of a host); *also* : a similar mass of filaments formed by some bacteria (as of the genus *Streptomyces*)

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